MEMORANDUM

Date: December 5, 2008

From: Alan Trounson, Ph.D., CIRM President

To: Independent Citizens Oversight Committee

Subject: Extraordinary Petition for Application RT1-01084

Enclosed is a letter from Dr. Juan Carlos Izpisua Belmonte, of the Salk Institute for Biological Studies, an applicant for funding under RFA 08-02, CIRM Tools and Technologies Awards. This letter was received at CIRM at least five working days prior to the December ICOC meeting, and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

As required by that policy, I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with Dr. Csete and the scientific staff, and concluded that the petition does not present compelling evidence that should alter the recommendation or score of the Grants Working Group (GWG). Briefly, the applicant included new data with this petition letter that was not previously included or cited in the application. CIRM staff recommends that the ICOC not consider data or information that was not made available to the GWG. The reviewers, as noted by the applicant were concerned about the feasibility of the approach presented in the application. In fact, the reviewers were unable to judge feasibility because the "Feasibility and (and where applicable) Preliminary Data" section, unlike the petition letter, contained only data with no attempt on the part of the applicant to discuss feasibility. In the future, the applicant may want to consider using the "Feasibility and Preliminary Data" portion of the application to make a case similar to that made in the petition letter. CIRM staff will be prepared to provide further analysis, should that be requested by any member of the committee.

Redactions, if any, have been made pursuant to the policy, in consultation with the author(s) of the letter. An unredacted version will be available for review in closed session.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

SALK INSTITUTE
FOR BIOLOGICAL STUDIES

Juan Carlos Izpisúa Belmonte, Ph.D.
Professor
Gene Expression Laboratory

Robert Klein, Chairman, Independent Citizen's Oversight Committee
Alan Trounson, President
Marie Csete, Chief Scientific Officer
California Institute for Regenerative Medicine
210 King Street, San Francisco, CA 94107

Re: Extraordinary Petition for ICOC Consideration of Funding Application RT1-01084-1

Dear Sirs and Madame,

I am writing to you in support of funding for our CIRM Tools and Technologies Award Application "Production of stable pluripotent selectable hES/iPS cell lines for site-specific integration of BAC reporter constructs." We are certainly very happy with the general positive expression from the reviewers on the broad impact that this proposal may have on the stem cell field as well as their enthusiasm for the strength of our research team. However, unwarranted concerns raised in the review apparently placed our application in a score range that is borderline for funding.

The main concern from the reviewers was the feasibility of the approach in human cells and the lack of demonstration of its utility in a model system (e.g. mouse ESCs). These concerns are not reasonable. One of our team members has not only published several key papers (Dev. Biol. (2007) 306:847-859; Nat Gen. (2005) 37(8):889-93; Cell. (2003) 113(3): 278-80; Genes & Dev. (2001) 15:2209-2214) on the BAC recombination approach but his methodology is currently being used by many labs in the field. Based on his expertise and to show further proof of principle of the approach presented in our CIRM proposal, we labeled different germline progenitors using BAC transgenesis in mouse ES cells. However, due to space constraints these data were not included in our original proposal, which was focused after all on studies in human. These data, shown on the following page, provide further support for our experimental approach.

We would greatly appreciate if this information would be provided to the ICOC members for their consideration in support of our application before making their final funding decisions for this RFA.

Sincerely yours,

Juan Carlos Izpisua Belmonte

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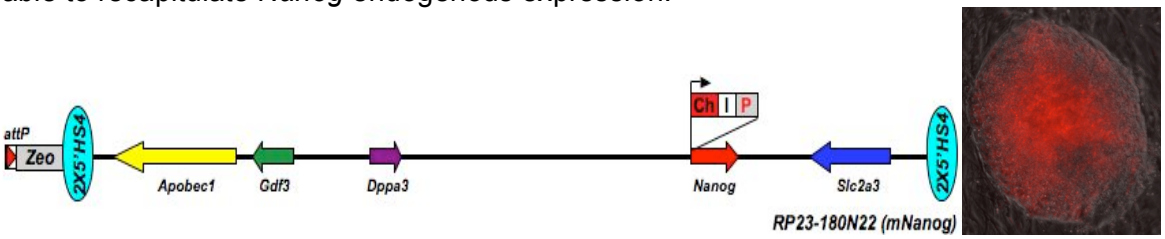
We have engineered a BAC (RP23-180N22) containing the *Nanog* using ET recombination. We deleted the *loxP* site located in the pBAC3.6 backbone and replaced it by a tandem repeat of the chicken betaglobin *5'HS4* insulator core sequence. With a second recombination, we targeted a mCherryIRESPuromycin cassette at the ATG of *Nanog*. A third ET-recombination allowed to delete the *lox511* site located on the other side of the insert, and to replace it by a tandem repeat of the chicken betaglobin *5'HS4* insulator core sequence linked to an *attPZeo* promoterless selection cassette. We electroporated this BAC in mouse ES cells, identified single insertion clones and characterized them. As shown in the picture bellow the transgene is able to recapitulate *Nanog* endogenous expression.



We have then used the genomic *attPZeo* cassette included in the *Nanog* transgenic clones to site-specifically insert an *Ngn3* reporter BAC transgene in ES cells using the *PhiC31* integrase. To achieve this we modified a BAC containing the *Ngn3* gene (RP23-121F10) using ET recombination. We first targeted an CeruleanIRESNeomycinFrtHygroFrt cassette at the ATG of *Ngn3* and then deleted the *lox511* site located 3' of the insert to replace it by a PGKPhiC31 constitutive expression cassette flanked by tandem repeat of the chicken betaglobin *5'HS4* insulator core sequence. We electroporated this construct in *Nanog-Cherry* ES cells and selected with *Zeocin*. 12 out of 12 Zeo resistant clones anlaysed were site-specific insertions of the *Ngn3* reporter BAC, as shown by PCR amplification with primers pairs (A-B) and (C-D), although one them (clone 2) seemed to have lost one extremity of the *Ngn3* BAC.